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# Prioritätsbescheinigung über die Einreichung einer Patentanmeldung

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**Aktenzeichen:** 103 04 100.1

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**Bezeichnung:** Process for producing a composition  
comprising carotenoids

**IPC:** "noch nicht festgelegt"

Die angehefteten Stücke sind eine richtige und genaue Wiedergabe der ursprünglichen Unterlagen dieser Patentanmeldung.

München, den 05. Februar 2004  
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**ABSTRACT**

A composition for delivering carotenoids (e.g.  $\alpha$ - and  $\beta$ -carotene, lycopene) and other active ingredients to the colon of humans after ingestion and for producing liquid food compositions insusceptible to polyphenol-protein reactions. This is achieved by encapsulating carotenoids with pectin, in particular with low-methoxylated pectin.



## PROCESS FOR PRODUCING A COMPOSITION COMPRISING CAROTENOIDS

### Field of the invention

The present invention relates to a composition comprising carotenoids and other active ingredients, in particular in encapsulated form.

### Background of the invention

Carotenoids have been reported to have beneficial effects on health. For example carotenoids such as  $\beta$ -carotene are thought to have an effect against colon carcinomas. It is believed that in order to have maximum beneficial effect, the carotenoids should be in such a (physical) form that they pass the stomach and small intestine without degradation or absorption, as it is believed to be desired for the effect on the colon that the carotenoids are available in the colon.

DE 19962427 discloses encapsulated bioactive components. The bioactive components that are mentioned are microorganisms with probiotic activity. The covering agent consists of non-digestible fibrous material. Examples given are: insoluble polysaccharides such as pectin, lignin, vegetable gums, but also soluble polysaccharides such as complex carbohydrates (e.g. fructo- or galactooligosaccharides, beta-glucans, etcetera). Example 6 mentions the use of a combination of pectin and inulin for encapsulating *Lactobacillus acidophilus*.

US 5,356,636 describes the preparation of vitamin or carotenoid products in powder form by preparing an aqueous dispersion of the vitamins and carotenoids concerned and film-forming colloids and reducing sugars, converting this dispersion into powder form, and thermally treating the powder. The gelatin is used in combination with organic amino compounds. The content of carotenoids is generally 5-50%.

US 4,389,419 discloses a process for encapsulating oils and oil-soluble substances (e.g. vitamin A) in microcapsules. The microcapsules are a shape-retaining alginate matrix filled with a precipitated polysaccharide and enclosing oil droplets.

EP 326026 discloses a method for reducing the oxidation of vitamins by combining the vitamins with triglycerides, complexing agents and covering agents, and optionally antioxidants. The covering agents disclosed are proteins, sugars, polysaccharides such as gum arabicum or starch.

EP 986963 describes a method for the production of a light- and oxidation-stable lycopene in the form of a dry powder. Said stability is achieved by having at least 20% of the lycopene in a crystalline form.

US 5,780,056 discloses microcapsules comprising a carotenoid and an edible oil. The coating material is based on gelatin. The microcapsules prevent the carotenoid from oxidation.

WO 91/06292 discloses a method for the production of water-dispersible microparticles containing e.g. carotenoids. The microparticles are prepared by a specific process involving milling in water with a hydrocolloid. The hydrocolloid is reported to be gelatin, gum arabicum, protein or starch.

DE 19637517 reports a method for preparing a particulate cold-water dispersible carotenoid preparation. This is achieved by making a dispersion of carotene, optionally oil and/or emulsifier, in an organic solvent, which dispersion is mixed with an aqueous solution of a colloid such as gelatin, starch, dextrin, vegetable protein, pectin, gum arabicum, casein.

Although the methods as reported may be suitable for some purposes, it was desired to have an alternative and improved preparation comprising carotenoids, which preparation should be such that the carotenoids are released in the colon, and not or to a limited extent in the intestinal tract preceding the colon. Although  $\beta$ -carotene may be the preferred active ingredient, the invention is also applicable to other carotenoids and to other active compounds, respectively.

#### Summary of the invention

It has now been found that the above objectives may be achieved (at least in part) by an edible composition comprising at least 25% wt (preferably at least 50% wt) of pectin and at least 0.2% wt carotenoid. Preferably, in the composition according to the invention the pectin has a degree of esterification below 50%. Such compositions can suitably be in the form of a particulate matter, preferably a dry particulate matter.

The above compositions may suitably be prepared by a process including the following steps:

- a) prepare an aqueous suspension comprising pectin (preferably with a degree of methoxylation below 50%),
- b) add a composition comprising carotenoid (at least 0.5%) and (vegetable) oil to the suspension under a) at a temperature of at least 55°C, preferably at least 60°C,
- c) emulsify the mixture at a temperature of 30-90°C, preferably at 50-65°C,

- d) spray emulsion of c) into a bath of an aqueous solution of calcium salts,
- e) separate the formed microparticles from the bath and optionally wash the microparticles
- f) optionally dry.

Alternative processes are possible for preparing formulations without oil.

#### Detailed description of the invention

It was surprisingly found that the composition as set out above is very well suitable to deliver the carotenoids where they are desired for the purpose. Carotenoids, when encapsulated in pectin, can pass the stomach and small intestine in a mostly intact form (i.e. more than 50% of the particles remain intact) and may reach the large intestine (the colon) without substantial uptake at earlier stages of the intestinal tract. Without wishing to be bound by theory, it is believed this is due to the properties of the pectin, which is hard to digest or to break down by the human body's own enzymes. It is thought that once in the colon, however, microorganisms that are present in the colon will produce enzymes which break down the pectic material. Such enzymes may be e.g. pectin lyases and pectate lyases and polygalacturonases. It is believed that pectin is quite unique in this respect, and as an additional advantage said pectin particles are relatively easy to prepare, and pectin is considered a well-accepted functional ingredient, especially in plant-derived food preparations.

The compositions according to the invention are preferably such that the carotenoid is encapsulated by the pectin. Such encapsulated carotenoids may be used as part of a food or dietetic composition, preferably in an aqueous composition. Examples of such liquid or viscous food compositions are fruit or vegetable juices and sauces/purees, (dietetic) fruit or vegetable drinks and (sports) drinks.

Preferably the composition according to invention comprise at least 1% (wt) carotenoid or other active compound. In principle all carotenoids may be used in the compositions according to the invention. Preferred carotenoids comprise  $\alpha$ -carotene,  $\beta$ -carotene, lycopene, astaxanthin, canthaxanthin, lutein, zeaxanthin, or mixtures thereof.

The compositions according to the invention may comprise further ingredients such as (vegetable or essential) oils in which the carotenoids may be dispersed or partly or completely dissolved. In such compositions the carotenoid may be dispersed in oil at a (wt) ratio carotenoid:oil between 1:500 and 1:2.

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Further additional ingredients may be compounds also exhibiting beneficial effects in the colon and which may prevent the stomach and small intestine from irritation and which should pass the stomach and small intestine without breakdown or digestion, respectively, or may be components that help stabilise the pectin/carotenoid composition, e.g. antioxidants, or components that are found together with carotenoids. A preferred antioxidant is  $\alpha$ -tocopherol, e.g. in an amount of 0.01-5% wt.

Compared to gelatin-based compositions, the use of pectin as encapsulating material according to the invention provides a further benefit when applied as an additive in fruit/vegetable juices or fruit/vegetable drinks with high fruit contents. Fruits contain a range of polyphenols, which in the presence of protein (e.g. gelatin) may interact to form a deposit of cloud, resulting in unattractive products. The use of pectin-based carotene compositions avoids protein-polyphenol-reactions and may therefore be suitable for producing attractive juices and drinks.

#### EXAMPLE

##### Example 1: preparation of $\beta$ -carotene-containing microcapsules.

For preparation of dry  $\beta$ -carotene-containing microcapsules a process was followed as given in Figure 1.

An aqueous suspension of 3% (wt) of low methoxylated pectin was prepared at 80°C using an Ultraturrax at 20.0000 rpm. A 30% dispersion of  $\beta$ -carotene in vegetable oil was added at a temperature of 55°C and under stirring, and the mixture was emulsified in an in-line Turrax at 15.000 rpm. The so-obtained emulsion was then sprayed into a calcium chloride solution. The formed microgel particles were separated using a centrifuge. After washing, the particles were freeze-dried to yield a red-coloured powder. Particle size: 60% wt has a size between 1 and 50 micron.

Sustained release of the active ingredient was tested by incubation of the microparticles with artificial gastric juice and with pectolytic enzymes at 35°C, and quantified by HPLC as described by MARX et al. (2000): *Food Chemistry* 70, pp. 403 – 408.

##### Example 2: $\beta$ -carotene-fortified orange juice.

Using the dried particles as prepared following example 1, a fortified orange juice may be prepared which contains 0.2-0.5 mg  $\beta$ -carotene / 100 ml to intensify the natural orange colour of the juice.

Particle sizes are preferably below 5 micron.

Example 3:  $\beta$ -carotene-fortified fruit drink.

An apple juice drink (juice content 25%) with up to 1 mg of carotene / 100 ml drink may be prepared using the dried particles as prepared following example 1.

This may be achieved by mixing the dried particles according to the invention (particle size preferably less than 5 micron) with water, sugar syrup, apple juice concentrate, lemon juice, ascorbic acid, and aromas. This mixture may be homogenised, pasteurised, degassed, filled into bottles and cooled. When kept in the dark at room temperature, no substantial undesired deposit will be formed within 12 months.

In this case the particles are used to yield both a colouring and a nutritional benefit.

After ingestion of this drink, the pectin wall of the particles will be broken down by the colon microflora and the carotene content will be released.

Comparative example

Example 3 may be repeated with a commercially available gelatin-containing  $\beta$ -carotene preparation (10% CWS  $\beta$ -carotene, ex Hoffmann-La Roche). Within several months a deposit will be formed by reaction of polyphenols from the apple juice with gelatin.

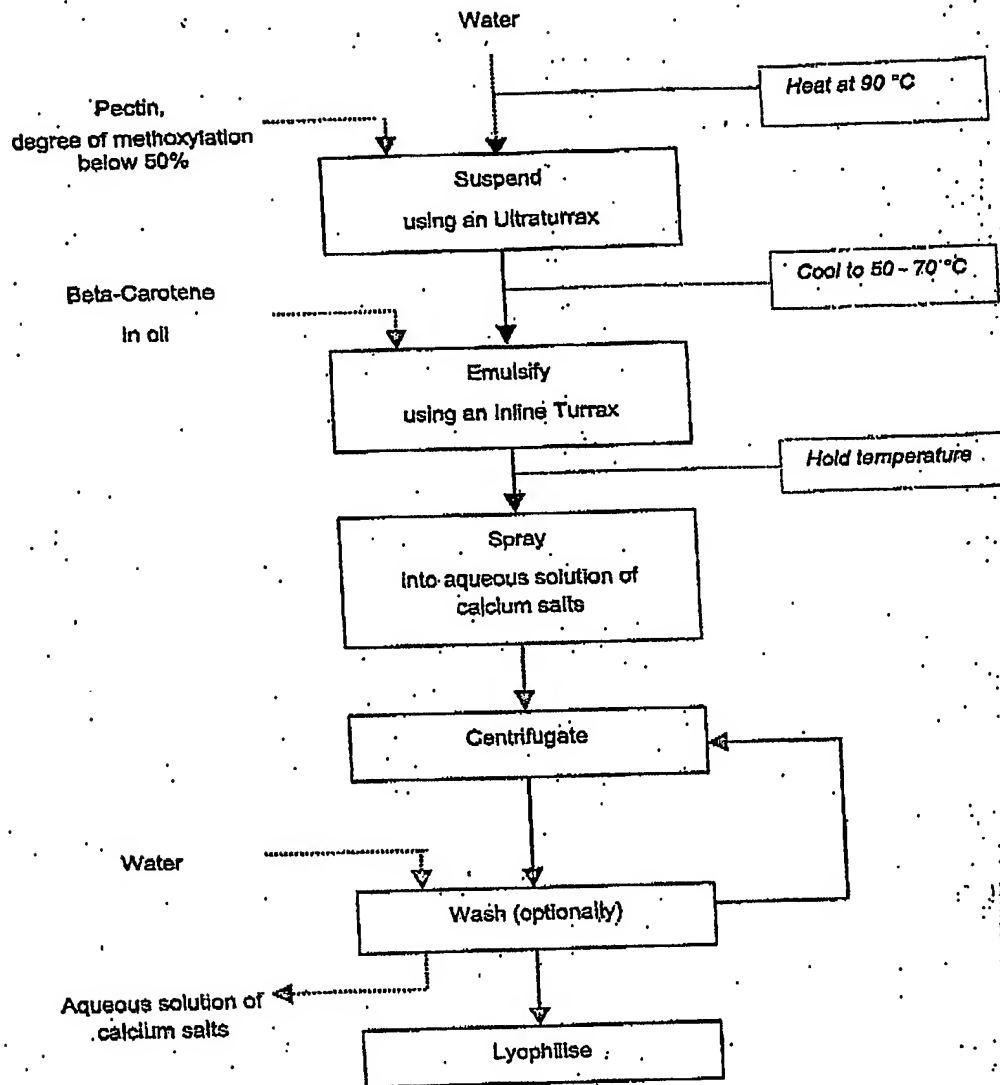
## Claims

1. Edible composition comprising at least 25% wt of pectin and at least 0.2% wt carotenoid.
2. Composition according to claim 1, comprising at least 50% wt of pectin.
3. Composition according to claim 1-2, wherein the pectin has a degree of esterification (DE) of below 50%.
4. Composition according to claim 1-3, wherein the pectin has a degree of esterification (DE) of between 30 and 45% and comprises between 60 and 75% of galacturonic acid.
5. Composition according to claim 1-4 in the form of a dry powder.
6. Composition according to claim 1-5, comprising at least 1% carotenoid.
7. Composition according to claim 1-6, wherein the carotenoid comprises lycopene,  $\alpha$ -carotene,  $\beta$ -carotene, astaxanthin, canthaxanthin, zeaxanthin, lutein, and mixtures thereof, respectively.
8. Composition according to claim 1-7, further comprising 0.01-5% antioxidant.
9. Composition according to claim 8, wherein the antioxidant comprises  $\alpha$ -tocopherol.
10. Composition according to claim 1-9, wherein the carotenoid is dispersed in oil at a (wt) ratio carotenoid:oil between 1:500 and 1:2.
11. Composition according to claim 1-9, wherein the carotenoid is completely or partly dissolved in oil at a (wt) ratio carotenoid:oil between 1:1000 and 1:5.
12. Composition according to claims 1-11, wherein the carotenoid is encapsulated by the pectin, in which „encapsulated„ also comprises „embedded„.
13. Food composition comprising the encapsulated carotenoid according to claim 12.



14. Food composition according to claim 13, wherein said food composition is a fruit juice or vegetable juice, sauce/puree, (dietetic) fruit or vegetable drink or (sports) drink.
15. Process for the preparation of a composition comprising carotenoids or other active ingredients, the process including the following steps :
- a) prepare an aqueous suspension comprising pectin,
  - b) add a composition comprising carotenoid (at least 0.5%) and, if necessary, oil to the suspension under a) at a temperature of at least 50°C, preferably at least 60°C,
  - c) emulsify the mixture at a temperature of 30-90°C, preferably at 50-65 °C,
  - d) spray emulsion of c) into a bath of aqueous solution of calcium salts,
  - e) separate the formed microparticles from the bath and optionally wash the microparticles,
  - f) optionally dry.
16. Process according to claim 15, wherein the pectin used is at least 50% low methoxylated pectin.

Figure 1.



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